Update from EFSA on GMO applications, mandates and other activities

Irina Olaru

9th GMO Network Meeting

8 November 2018

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European Food Safety Authority

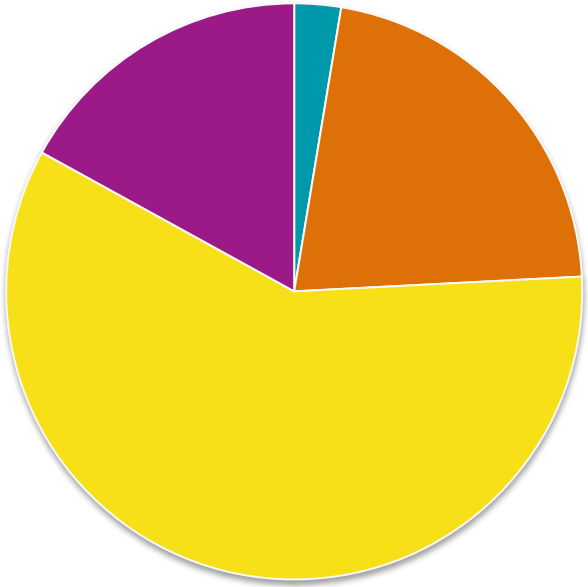
EFSA ACTIVITIES ON GMO

- Applications under 1829/2003
  - Under Articles 5 and 17
  - Renewals under Articles 11 and 23
- Guidance documents and explanatory notes
- External mandates
- Procurement and grants

APPLICATIONS

APPLICATIONS UNDER 1829/2003, ART 5 & 17 – TOTAL: 153

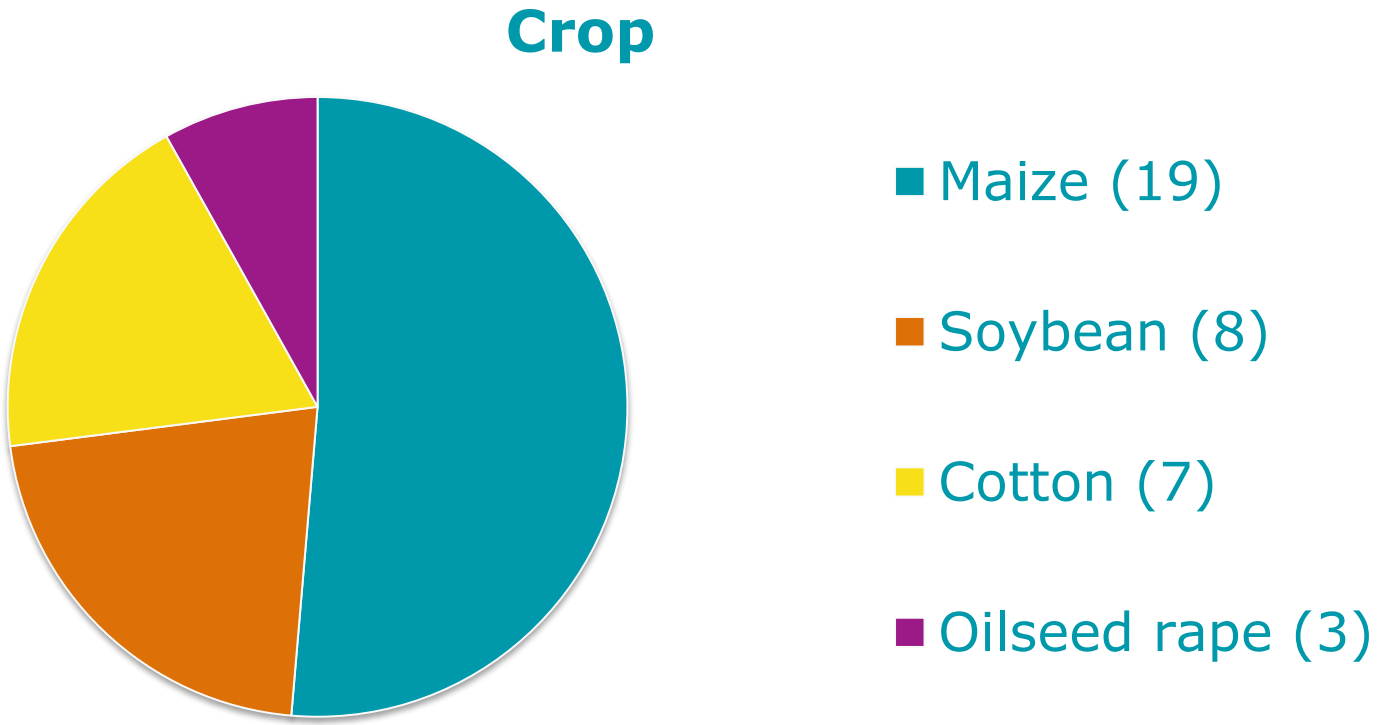
Status



- Completeness check (4)
- Risk assessment (33)
- Finalised (90)
- Withdrawn (26)

APPLICATIONS

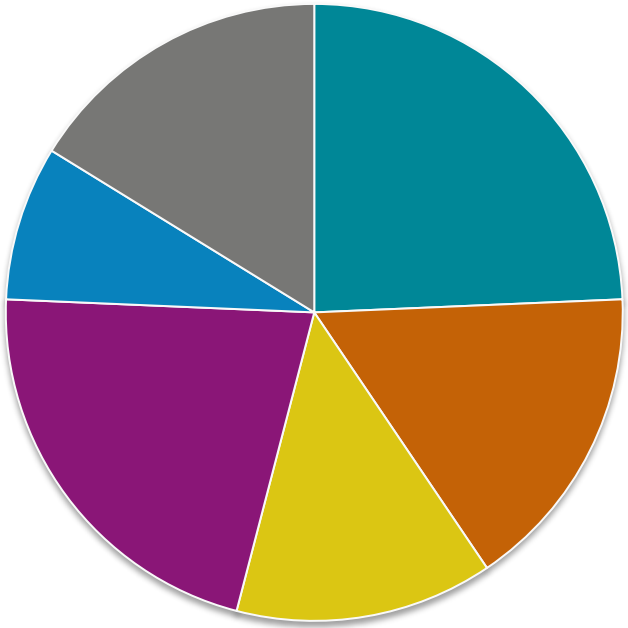
APPLICATIONS UNDER 1829/2003, ART 5 & 17 – CC+RA (37)



APPLICATIONS

APPLICATIONS UNDER 1829/2003, ART 5 & 17 – CC+RA (37)

Level of stacking



- Singles (9)
- 2-event stacks (6)
- 3-event stacks (5)
- 4-event stacks (8)
- 5-event stacks (3)
- 6-event stacks (6)

APPLICATIONS

Renewal applications under 1829/2003, Art 11 & 23

TOTAL: 15

- Finalised: 9
- In completeness check: 3 (RX-013 maize MIR604; RX-014 maize MON 88017; RX-015 maize MON 89034)
- Under risk assessment: 3 (RX-02 OSR GT73; RX-09 soybean A2704-12; RX-012 OSR T45)

GUIDANCE DOCUMENTS AND EXPLANATORY NOTES

Recently finalised

- Technical Note on the quality of DNA sequencing (item 4.2)
- Explanatory note on the selection of forage material (item 4.3)
- Explanatory note on the determination of newly expressed protein levels (item 4.4)

GUIDANCE DOCUMENTS AND EXPLANATORY NOTES

Under development

- Human dietary exposure assessment to endogenous and new constituents in GM foods (item 4.5)
- Update of the Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual PMEM reports on GMOs authorised in the EU market – foreseen for the first quarter of 2019
- Submission guidance for renewal applications – to be published by the end of 2018

EXTERNAL MANDATES

Gene drive

- **Terms of Reference**: EFSA is requested to identify potential risks in terms of impact on human and animal health and the environment, and potential novel hazards, and to determine whether the existing guidelines are adequate or whether there is a need for updated guidance. Under the present mandate, EFSA is not requested to develop guidelines for the RA of gene drive modified organism.
- EFSA will start with the organisms most likely to be release in the near future (i.e. insects); an ad-hoc WG will be established.
- **Deadline**: 31 March 2020 (draft).

EXTERNAL MANDATES

Synthetic biology

- **Terms of Reference**: Main objective of the mandate is to determine whether the existing guidelines are adequate or whether there is a need for updated guidance. The activity will be divided in two: plants for environmental release (lead by the GMO Panel) and microorganisms (lead by EFSA's Scientific Committee).
- Ad-hoc WG of the GMO Panel and the Scientific Committee will be established.
- **Deadline**: 31 March 2020 (draft)

EXTERNAL MANDATES

- MON810 PMEM annual reports – the last report received and assessed is for 2016
- Additional information on maize 3272 (Application 34) – received in April 2017, on-going
- Scientific assistance on Santos-Vigil et al 2018 regarding the allergenic potential of Cry1Ac
- Sequencing mandates: 12 finalised, 2 on-going (soybean A2704-12 and DAS-81419-2)

PROCUREMENT AND GRANTS

Contractor support on:

- Statistical analyses
- Toxicological analyses

- Literature review of baseline information on RNAi that could support the food/feed and environmental risk assessment of RNAi-based GM plants – finalised, online report
- Refined Protocol for in vitro digestion of Proteins for allergenicity assessment – on-going
- Literature review in support of adjuvanticity/immunogenicity assessment of proteins – on-going

Thank you for your attention!

Questions?

EFSA Consultation of national Competent Authorities on GMO applications

-

Reporting and Discussion

Annual meeting with GMO Network of Member States
Parma, Italy – 8 & 9 November 2018

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INTRODUCTION

Scope

To engage a dialogue with the national Competent Authorities on the 3-month commenting period on GMO applications

Outline

- Reporting past experiences
- Taking stock of the situation
- Discussing possible way(s) forward

REPORTING PAST EXPERIENCES

Remit Applications for import and processing for food and feed uses under Regulation (EC) No 1829/2003

Legal basis Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003: *National competent authorities (CAs) have three months to make their opinion known to EFSA*

Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003: *EFSA opinion, including opinions from CAs, is made publicly available*

REPORTING PAST EXPERIENCES

Over time we have identified different ‘categories’ of comments, some of them are raised recurrently irrespective of the application at stake:

- Comments falling outside the remit of EFSA, i.e. risk assessment
  - E.g. Detection method, labelling proposal
  - E.g. Practical implementation of PMEM/GS plans
- Comments falling outside the remit of the GMO risk assessment
  - E.g. Residues of pesticides
- ‘Statements’ rather than comments raising strong disagreements without supporting reasoning/evidences

REPORTING PAST EXPERIENCES

On the contrary, the following comments are of major importance and contributed significantly to the risk assessment of GMOs by the GMO Panel:

- Comments identifying missing information (e.g. study reports),
- Comments supported by science-based reasoning/evidences,
- Comments reporting lack of compliance with guidelines in place (without questioning the guidelines *per se*)

THANK YOU !

TAKING STOCK OF THE SITUATION

Our conclusions from past years' experience

- The remit of EFSA and its MS consultation needs clarifications,
- The Panel/Unit takes into account all comments related to risk assessment (RA), and
- The Panel/Unit addresses all these comments in both, the scientific opinion and Annex G to EFSA opinion.

Some room for improvement…

- Overall to optimize the use of respective resources
  - To focus on technical/scientific comments of added value for the RA
  - To lean the 'reporting' process without undermining the level of scrutiny of the comments
- Still to maintain compliance with the legislation

DISCUSSION ON POSSIBLE WAY(S) FORWARD

Scenarios		Pros	Cons
Status quo	**CAs** Range of comments beyond RA **EFSA** Double reporting		-Poor use of mutual resources -Likelihood to overlook comments relevant for RA
	CAs Clear scope of consultation, i.e. RA-related comments only	- Added value for RA - Improved use of resources (for both CAs and EFSA)	
	EFSA Addressing and reporting on RA-related comments only, in SO and Annex	- Added value for RA - Improved use of EFSA resources (owing to clear scope)	-Time-consuming screening of comments - No efficient use of resources (owing to reporting)
	EFSA Addressing and reporting on RA-related comments in SO only	-Improved use of resources (for EFSA) -No loss of info - Compliant with legislation	


Thank you for your attention
Your views ?
Discussion is open!
EFSA

90-day studies on the whole GM food/feed under Reg.(EU)503/2013

EFSA Implementation Strategy

Anna Lanzoni

Senior Scientific Officer, GMO Unit

Annual meeting with GMO Network of Member States
Parma, Italy – 8 & 9 November 2018

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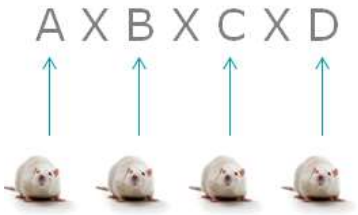
Legal requirements

- 90-day study on single-event dossiers

mandatory

- 90-day studies in stacked-event dossiers

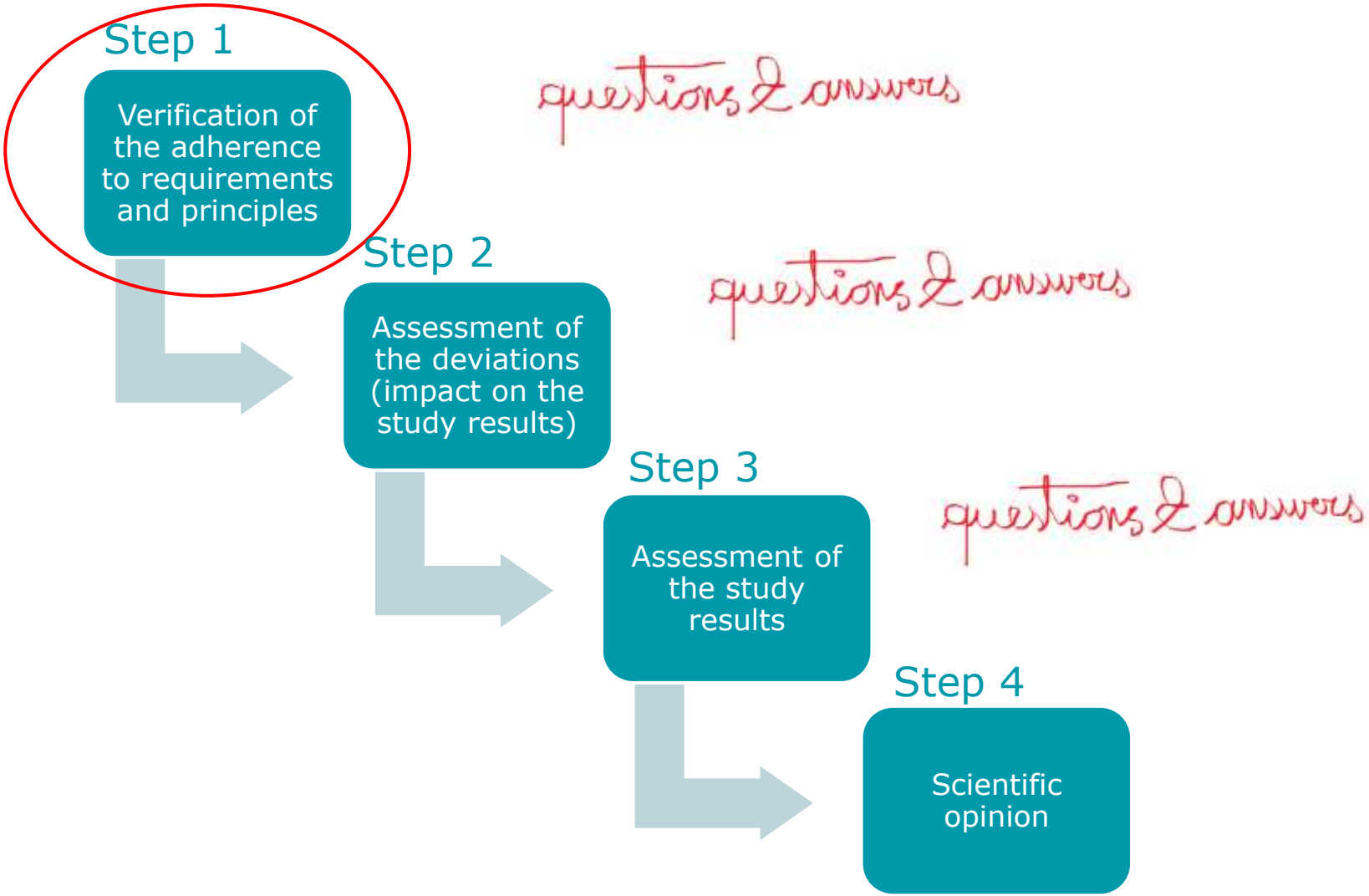


mandatory
- new
- previously provided & in line with Reg. (EU)503/2013

hypothesis-driven

Reg. (EU) No. 503/2013 (Annex II, II, 1.4.4.1)
ARES (2013) 3227887 11/10/13, ARES (2014) 1155663 11/4/2104

Risk assessment by EFSA– the flow



Risk assessment by EFSA

- Detailed scrutiny to check for the adherence to the requested frame

  - ✓ Reg.(EU) 503/2013
  - ✓ OECD TG408
  - ✓ GLP
  - ✓ EFSA SC 2011
  - ✓ EFSA 2014

EFSA (European Food Safety Authority), 2014.
Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal 2014;12(10):3871, 25 pp., doi:10.2903/j.efsa.2014.3871

- Framework contract in place

Checklist

1	**OECD TG408/EFSA Guidance docs**
2	**GLP statement**
3	**Test item**
4	**Control material**
5	**Test/control diets**
6	**Test system**
7	**Dose groups and dose level selection**
8	**Measurements and observations**
9	**Experimental design**
10	**Raw data**

Checklist

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10	**Raw data**

Test & control materials
Test & control diets

- Identity
- Pedigree (similarity CC vs GM)
- Treatment with the intended herbicide (HT)
- Source and chain of custody
- Stability
- Homogeneity and concentration

Checklist

1	OECD TG408/EFSA Guidance docs
2	GLP statement
3	Test item
4	Control material
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6	Test system
7	Dose groups and dose level selection
8	**Measurements and observations**
9	Experimental design
10	Raw data

Missing histopathology

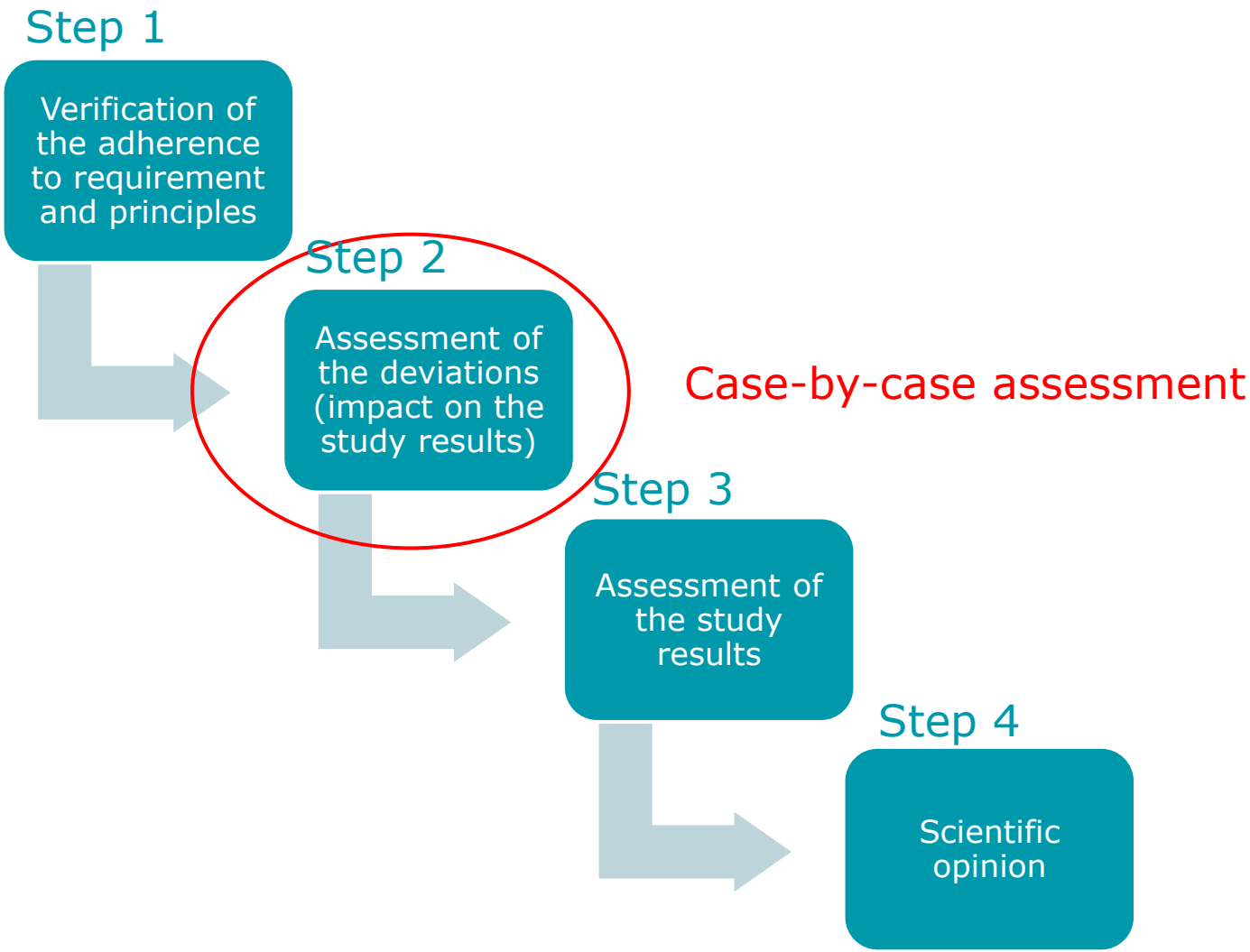
Checklist

1	**OECD TG408/EFSA Guidance docs**
2	**GLP statement**
3	**Test item**
4	**Control material**
5	**Test/control diets**
6	**Test system**
7	**Dose groups and dose level selection**
8	**Measurements and observations**
9	**Experimental design**
10	**Raw data**

Experimental design, Statistical analysis

- Randomisation procedure
- Cage disposition
- Missing data
- Cage effects (social housing)
- Flowchart (statistical analyses and the outputs obtained)
- Outlier checks, data transformation and choice of model
- Goodness-of-fit
- Full output of the models

Risk assessment by EFSA– the flow



90-day studies on single events in stacks

The situation

- Several spontaneously submitted by applicants in the context of singles
- None impacted the RA conclusion of the single event GMO
- Many completed before EFSA SC, 2011
- Some containing weaknesses with regard to OECD TG 408 and/or EFSA guidance documents (described in SOs)

90-day studies on single events in stacks

The assessment

To fulfil legal requirements:

- EFSA checks for study ADHERENCE to Reg. 503/2013 and relevant EFSA guidance documents
- Deviations are discussed and questions to applicants can be asked.
- Additional information received are integrated in the stack SO.

Critical weaknesses

Study design

- Low number of exp. units
- GM plant material not treated with the intended herbicide(s)
- Low dose tested

Data analysis

- Missing histopathology

Procedural considerations

Pertaining to stacks applications

- Stand-alone dossiers
  - Redundant questions under different applications
  - Stepwise submission of studies accepted
  - Additional info is 'processed' as soon as received, irrespective of the dossier
- Submission of additional info
  - By the requestor only
  - Applicants requested to inform EFSA of delayed and anticipated submission
- If new study is required, clear identification (study report number) and where to find it

Thank you!

Questions?

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Stacks - Further clarifications

Ref. Ares(2014)1155663 - 11/04/2014

EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL

Deputy Director General for the Food Chain

Brussels,
SANCO/E1/SP/mb sanco.ddg2.e.1(2014)1140685

Dear Mr Späth,

Subject: **Outstanding issues related to Commission Implementing Regulation (EC) No 503/2013**

90-day studies required for single events

The requirement to include single events data within a stack application does not put into question the validity of already authorised single events even when no 90-day study was originally provided, since these single events will still be covered by their original authorisation until the end of the validity period. This requirement has the only objective to cover the risk assessment of stacks which is primarily based on the risk assessment of single events constituting it and not to re-authorise these single events.

Therefore as explained in our letter of 11/10/2013, in case of stack transformation events, a 90-day feeding study with whole food/feed has to be submitted for each single event constituting the stack when not previously provided or not in line with the principles outlined in the Regulation.

Technical Note on Sequencing quality

GMO Network meeting, 8-9 November 2018

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TECHNICAL NOTE TO SEQUENCING QUALITY

- **EC Mandate** Technical note to applicants on, and checking of, the quality of the methodology, analysis and reporting covering full sequencing and insertion site analysis of GM event, and generational stability and integrity

- **State of play** EFSA Technical Note published (July 2018)

- **Implementation** From 1 October 2018 EFSA takes over from JRC, the DNA sequencing quality check in GMO dossiers.

PURPOSE OF THE TECHNICAL NOTE ON DNA SEQUENCING QUALITY

- To support the applicants and MS risk assessors
- To harmonise the risk assessment of GMOs
- To harmonise the submission of data when DNA sequencing is used and reflect the scientific progress of the methodology used
- To provide quality parameters and requirements, or discuss considerations, when DNA sequencing is used for parts of the molecular characterisation:
    a. Sequencing of the insert(s) and flanking regions, building on the 2016 JRC guidelines;
    b. Determination of the number of inserts;
    c. Genetic stability, in singles and stacks

MC ASPECTS FOR WHICH DNA SEQUENCING IS USED:

- Sanger sequencing is currently used:

a. for the characterisation of the insert(s) and flanking regions

b. for the demonstration of genetic stability across generations

- Next generation sequencing (NGS) can be used:

a. for the characterisation of the insert(s) and flanking regions

b. for the determination of the number of all detectable inserts

c. for the genetic stability and integrity

OUTLINE OF THE TECHNICAL NOTE ON SEQUENCING QUALITY

1. Introduction
2. Data and Methodologies
3. Material and sample preparation
4. General requirements for the sequencing quality when Sanger sequencing is used in GMO applications
5. General considerations on the quality parameters for NGS reads when NGS is used in GMO applications
6. Sequencing for the characterisation of the insert(s) and flanking regions
7. Determining the number of all detectable inserts
8. Genetic stability
9. Data format requirements

MATERIAL AND SAMPLE PREPARATION

- General requirements on the sample information used for sequencing:

  - Source of material (GM plant, tissues)
  - Breeding tree
  - Number of individuals used for the material collection
  - Report on sample preparation (DNA extraction protocol, overall strategy)

GENERAL REQUIREMENTS FOR SANGER SEQUENCING

- Based on the current JRC guidelines:
  - Two independent PCR reactions
  - Bi-directional sequencing
  - Two independent sequencing experiments
  - The raw sequence of each nucleotide should be covered 4 times
  - A full sequencing report on: strategy, details of experiment, description of the sequencing method, experimental design
  - Specific requirements for sequence format and alignments
  - Information on any manual editing performed (base–calling, trimming); to be reported and justified.

GENERAL REQUIREMENTS FOR NGS

This part describes the most relevant parameters that will be considered when NGS methodology and generated datasets are assessed in applications.

a. Quality of datasets

b. Library preparation and sequencing strategy

c. Coverage (average read depth)

d. Description of bioinformatics (flowchart of analysis)

SEQUENCING OF THE INSERT(S) AND FLANKING REGIONS

- Specific considerations when addressed by Sanger sequencing
- Specific considerations when addressed by NGS
- A combination of approaches, including using longer reads such as PacBio, sequencing of cloned genomic fragments or PCR amplicons (including by Sanger) may be needed in cases where the configuration of the inserted sequences is more challenging.

DETERMINING THE NUMBER OF ALL DETECTABLE INSERTS

- To detect junction reads: partially matching both insert and host genome (chimeric) → identify junctions

- Information on genome coverage (specific for Junction Sequence Analysis)
  - Depending on the genome
  - Depending on the sequencing technology used

- Justification by the applicant

GENETIC STABILITY

- Single events: NGS as alternative to currently used methods. This can be accomplished with an analysis of the insert sequence with flanking regions by mapping of NGS reads (or contigs) to the final sequence (of the insert and the flanking regions) over multiple generations.

- Stacks: Regulation (EU) No 503/2013 requires the sequence of the events in the stack to be determined and compared to that of the single event. This can be achieved by Sanger or NGS.

DATA FORMAT REQUIREMENTS

- Final sequence in ASCII text files: must be annotated according to INSDC Feature Table Definition document.
  - Including specific descriptors and features

- Specific requirements for Sanger experiments:
  - ABI or FASTQ format
  - All sequences must be aligned to generate a consensus sequence or final sequence.
  - Alignment in CLUSTAL or FASTA format

- Specific requirements for NGS experiments:
  - Raw NGS reads in FASTQ format
  - Aligned mapped sequences provided in SAM, BAM or CRAM format
  - Additional ACE file suggested to be submitted

ANNEXES

- Annex 1

Checklist for Compliance check by the applicant

- Annex 2

Structure of the Sequencing folder and files for submission

SUPPORTING FILES

Supporting Information

Filename	Description
efs25345-sup-0001-Annex-01.pdf PDF document, 548.4 KB	List of information to be submitted to EFSA for each GM event, according to the Technical Note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants
efs25345-sup-0002-Annex-02.pdf PDF document, 226.9 KB	Instructions to organise the sequencing information to be submitted to EFSA in accordance with the Technical Note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants
efs25345-sup-0003-SequencingInfo.zip Zip archive, 5 KB	Sequencing Info

Please note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing content) should be directed to the corresponding author for the article.

IMPLEMENTATION

- Applicable to new dossiers: from October 2018
- EFSA sequencing quality/compliance check: by APDESK & GMO Unit
- Data storage: DMS
- 'Oversized' GMO data files, e.g. NGS raw data:
  - Set up of GMO space in EFSA cloud (DTS).
  - Ongoing progress: setting up secure remote access (VPNs); to provide instructions & information to GMO network members.
- WEBINAR: TBD (early 2019)

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Clarifications on the data submitted

- One report (one PDF) describing material and sample, sequencing methodology and bioinformatics analysis, etc.
- One Annex 1 per event, including all apsects where sequencing has been used.
- If different DNA extraction methodologies are used, all should be provided;
- Full description along with the script, source code information and pipelines should be provided;
- 40x coverage for NGS experiments was used as an example in the technical note;
- Both insertion sites and the structure of the insert need to be demonstrated for genetic stability over several generations.

Explanatory note on the selection of forage material suitable for the risk assessment of GM feed of plant origin

Annual meeting with GMO Network of Member States

Parma, Italy – 8 & 9 November 2018

Michele Ardizzone – Officer in GMO Unit

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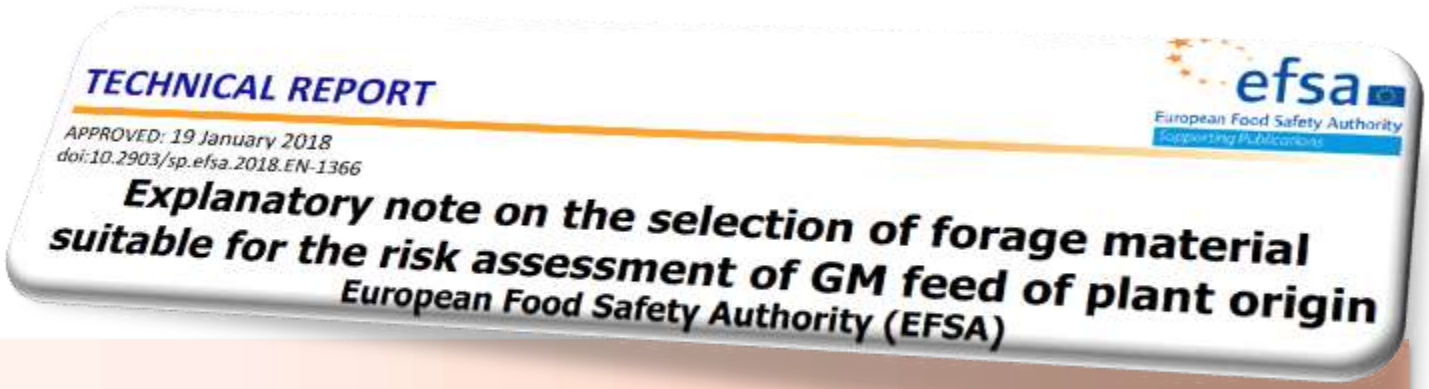
Explanatory note: purpose



Scope:

To harmonise and support the applicant in the selection of forage material suitable for the risk assessment of GM feed of plant origin

Structure of the document:

- Regulatory provisions relevant for the GM feed risk assessment
- Need of a definition of forage

- Definition of forage
- Criteria for selecting appropriate forage material (RA of GM feed)
  - maize, soybean, rapeseed, cotton and sugarbeet

Regulatory provisions relevant for GM feed risk assessment

COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013
of 3 April 2013
on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006

Information on the expression of the insert(s)

- The applicant shall provide information to demonstrate whether the inserted/modified sequence results in intended changes at the protein, RNA and/or metabolite levels. **Data on expression levels from those parts of the plant used for food and feed purposes** shall be provided in all cases.

Exposure assessment — Anticipated intake/extent of use

- The applicant shall **determine** by appropriate methods **the concentrations** of the newly expressed proteins, other new constituents and endogenous food and feed constituents, of which the levels have been altered as a result of the genetic modification (for example, due to changes in metabolic pathways) **in those parts of the genetically modified plant intended for food or feed use.**

Selection of material and compounds for analysis

- Unless duly justified, **analysis shall be carried out on the raw agricultural commodity,** as this usually represents **the main point of entry of the material into the food and feed production and processing chain**.

Comparative analysis of composition

- **Analysis of plant cell wall components are also required for the vegetative parts of plants used for feed purposes.**

Parts of the GM plants "intended for feed uses"

GM feed of plant origin

- **Raw agricultural commodities**
  - grain, bean, seed and root
  - forage
- **Processed commodities**
  - meal, cake
  - gluten meal, gluten feed

Need of a definition of forage

- Grain, bean, seed and root identify feed material belonging to well-defined parts of the plant
- Forage is a general term to identify feed material but, *"per se"* does not refer to specific parts of the plant

  → This can create ambiguity when collecting data for the risk assessment of GM feed

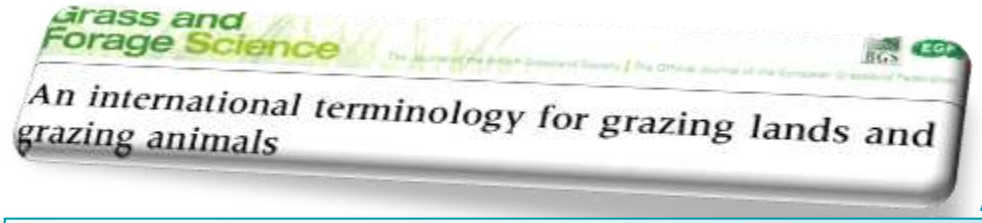
- **Lack of suitable definition of forage in regulatory and OECD context was confirmed**
  - GMO EU legislation:
    - Reg. (EC) No 1829/2003
    - Reg. (EU) No 503/2013
  - Non-GMO EU legislation (food/feed risk assessment):
    - Reg. (EC) No 178/2002
    - Reg. (EC) No 767/2009
    - Reg. (EU) No 2017/1017
  - OECD Consensus documents on compositional considerations for new plant varieties
    - maize, soybean, sugarbeet, rapeseed, cotton

Definition of forage suitable for the RA of GM feed

Besides regulatory provisions, a crop-specific* definition of forage was given, based on:

- Scientific literature
- Current animal feeding practice
- Current agricultural practice

*** maize, soybean, sugarbeet, rapeseed, cotton**



Allen et al, 2011

Forage defined as the edible parts of plant, other than separated grain, that can provide feed for grazing animals, or that can be harvested for feeding, conserved in situ or preserved and store.

Current animal feeding practice

Current agricultural practice

Criteria set for collection of appropriate forage material

MAIZE

Uses

- Grains: human food, animal feed, ethanol production and non-food products
- Whole aerial plant: forage for animals

Agro-zootechnical practices

Harvest for collection of mature grains - stage BBCH87 (R6)

- whole aerial plant left after grain removal is shredded to obtain forage, consisting of stalks, leaves, cobs and husks.

Harvested for collection of whole aerial plant (including grain) - stage BBCH85 (R4)

- whole aerial plant is shredded to obtain forage consisting of stalks, leaves, cobs, husks and grains

Criteria set for collection of appropriate forage material

SOYBEAN

Uses

- Oil- and protein-rich beans (full fat beans), processed to obtain vegetable oil for human food, animal feed and energy source
- Whole aerial plant: may be fed to animals as forage

Agro-zootechnical practices

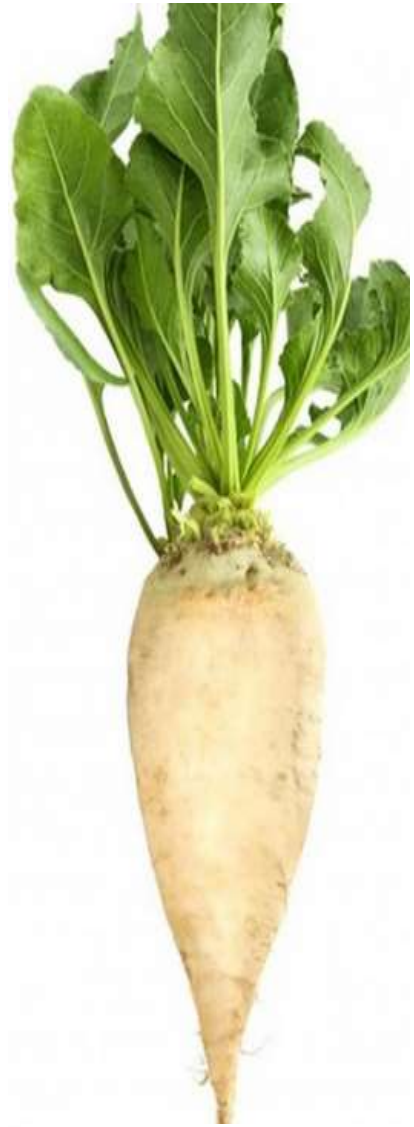
Harvested for collection of mature beans - stage BBCH87 (R6)

- whole aerial plant left after bean removal is shredded to obtain forage, consisting of stems and leaves

Harvested for collection of whole aerial plant (including beans) - from flowering to stage BBCH87 (R6)

- whole aerial plant is shredded to obtain forage consisting of stems, leaves, husks and beans

Criteria set for collection of appropriate forage material



SUGARBEET

Uses

- Mainly used for sugar production
- roots are occasionally fed to dairy cattle and pigs
- tops (leaves and crowns) may be fed to cattle as forage

Agro-zootechnical practices

Harvested at maturity - stage BBCH49

Criteria set for collection of appropriate forage material

RAPESEED

The use of rapeseed and cotton plants as forage is currently not a common practice

COTTON

Summary of the criteria for selecting forage material

Crop	Forage material
Maize	Mixture of stalks, leaves, cobs and husks, excluding grains (e.g. stage BBCH87) and/or Mixture of stalks, leaves, cobs and husks, including grains (e.g. stage BBCH85) An explanation of the rational followed to sample forage material should be provided.
Soybean	Mixture of stems and leaves, excluding husks and beans (e.g. stage BBCH87) and/or Mixture of stems and leaves, including husks and beans (any stage from flowering to maturity could be selected). An explanation of the rational followed to sample forage material should be provided.
Sugarbeet	Tops (mixture of leaves and crowns) (stage BBCH49)
Rapeseed	The use of rapeseed forage is currently not a common practice, and the OECD consensus document does not include a list of key constituents for forage. Data from rapeseed forage are not considered necessary to assess the safety of the commonly used rapeseed feed materials.
Cotton	The use of cotton forage is currently not a common practice, and the OECD consensus document does not include a list of key constituents for forage. Data from cotton forage are not considered necessary to assess the safety of the commonly used cotton feed materials

Thank you for your attention

Your views ?
Discussion is open!

Explanatory note on the determination of Newly Expressed Protein levels

Annual meeting with GMO Network of Member States
Parma, Italy – 8 & 9 November 2018

efsa
European Food Safety Authority

EFSA GD 2011

- **Information on the expression of the inserted/modified sequence :**
  - The applicant should provide information to demonstrate whether the inserted/modified sequence results in intended changes at the protein, RNA and/or metabolite levels. In many cases the intended genetic modification will lead to **the expression of new protein(s)**, therefore **protein expression data will be the most relevant**.
  - The applicant should present the following information:
    - **methods** used and the **raw datasets**. The **specificity** of the protein analysis method should be **demonstrated**
    - if the insert encodes new protein(s), the **range and mean values** for the levels of the newly produced protein(s)

- **Toxicological assessment**: The toxicological impact of any biologically relevant change in the GM plant and/or derived food and feed resulting from the genetic modification (e.g. **expression of introduced genes**….) should be assessed

- For GM plants containing **stacked events** …. the **main objective** of the analysis is **to assess the potential for any interactions** between the events which may raise safety concern. If **newly expressed proteins** are present in the GM plant, their **levels should be compared**……

Regulation (EU) 503/2013

- EXPOSURE ASSESSMENT — ANTICIPATED INTAKE/EXTENT OF USE

An estimate of the expected intake shall be an essential element in the risk assessment of genetically modified food and feed and shall also be required for the nutritional evaluation. Information shall be provided by the applicant on the intended function, the dietary role, and the expected level of use of the genetically modified food and feed in the EU. In addition, **the expected range of concentrations of newly produced proteins** or existing plant proteins deliberately modified in the genetically modified food(s) and feed(s) to be placed on the market **shall be provided**.

Use of Newly Expressed Protein (NEP) levels data in GM plant RA

- According to relevant EFSA GMO Panel GD and legislation for GM plant RA, information on NEP levels is needed for:
  - Molecular characterisation of the event(s)
  - Assessment of the exposure to these NEPs in the context of food/feed and environmental safety
  - Interactions in stacked events affecting NEP levels
- No details on how to produce these data are included
- Self task of EFSA to 'fill this gap'

Terms of reference and data/methodologies

- An explanatory on NEP levels determination to provide details on key methodological aspects to be considered by applicants in order to harmonise the information in submitted GM plant applications
- Based on:
  - EFSA GMO Panel guidelines on Food & Feed and ERA for GM plants
  - relevant legislation (e.g. Regulation (EU) 503/2013)
  - other bioanalytical method validation documents
  - scientific literature
  - gained experience from already assessed EFSA GM plant applications
- MC WG was consulted and input also received from F&F WG
- No specific protocols are recommended

Content

- Two aspects:
  - protein extraction
  - analytical method for NEP quantification
- Main methods discussed (ELISA, WB and MS) with particular attention to ELISA (most widely method used)
- Recommendations on what/how information should be reported (checklist)

Protein Extraction

- Efficiency
  - total NEP levels are needed for the RA; chosen method might have limited 'extractability'
  - a 'complete' extraction step under strong denaturing ('harsh') conditions to determine the remaining NEP amount in the 'insoluble' fraction
  - NEP extraction efficiency estimated relative to total NEP amount
- Tissue disruption/cell lysis (NEP molecular stability)
  - lyophilised material
  - appropriate buffer-to-tissue ratio
- Extraction buffer (NEP molecular stability)
  - buffer compatible with quantification method
  - protease inhibitors should be used; if not justification should be provided

Analytical method (NEP quantification)

- Validation parameters (and data quality considerations)
  - Sensitivity; LOD, LOQ, standard curve
  - Matrix effects; method should be sufficiently accurate in the presence of tissue matrix components (for every tissue analysed)
  - Specificity‘; e.g. antibody cross reactivity for ELISA/WB, peptide interference for MS-based methods)
  - Repeatability; variation in inter-assay and intra-assay measurements, CV

Analytical method (NEP quantification)

- Additional elements, e.g.:
  - Information on the reference standards (e.g. full length proteins/equivalence, IS for MS)
  - Antibody information (monoclonal/polyclonal, antigen used)
  - MS-specific (e.g. digestion efficiency)
  - Processed food/feed (e.g. choice of antibodies)

Presentation of results

- Information on the samples e.g.
  - number of analysed plants and how plant material was collected
  - Sufficient description of plant/tissue treatment (herbicides)
  - any sample contamination and impact on results
- Information on the methods, e.g.
  - Method description (protocols)
  - all critical methodological parameters
- Data analysis and reporting, e.g.
  - % extraction efficiency should be applied (and described)
  - presented values in both FW and DW (conversion should be explained)
  - all acceptance criteria should be described and justified and the impact of data falling out of these criteria should be discussed

Transition period

- In line with the indicative timelines for submitting molecular datasets that require the generation of plant material, the recommendations will be applicable for GM plant applications submitted **24 months** after its publication
- However, applicants are recommended to take into account the elements described in this document for all applications submitted after its publication
- A transition period of **two months** applies to those elements for which only the provision of information is recommended


Thank you for your attention
EFSA

Human dietary exposure for the risk assessment of GM foods

Annual meeting with GMO Network of Member States

Parma, 08.11.2018

efsa
European Food Safety Authority

- **Dietary exposure in the risk assessment of GM foods**
- **How to estimate dietary exposure**
- **Human dietary exposure: current situation**
- **Future**

- **Dietary exposure in the risk assessment of GM foods**

- How to estimate dietary exposure

- Human dietary exposure: current situation

- Future

DIETARY EXPOSURE AS PART OF THE RA OF GM FOOD

LEGAL

IR 503/2013

2. EXPOSURE ASSESSMENT — ANTICIPATED INTAKE/EXTENT OF USE

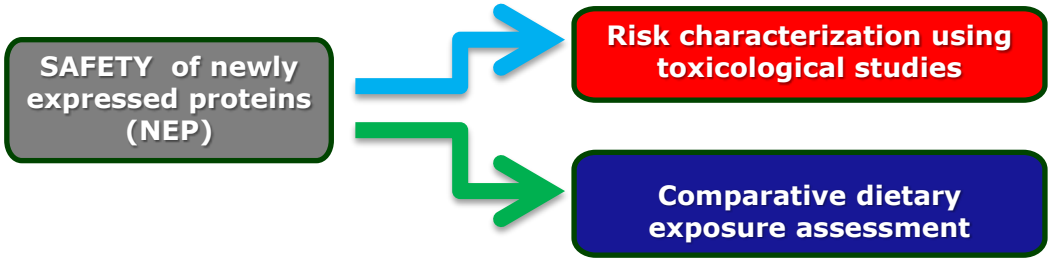
An estimate of the expected intake shall be an essential element in the risk assessment of genetically modified food and feed and shall also be required for the nutritional evaluation. Information shall be provided by the applicant on the intended function, the dietary role, and the expected level of use of the genetically modified food and feed in the EU. In addition, the expected range of concentrations of newly produced proteins or existing plant proteins deliberately modified in the genetically modified food(s) and feed(s) to be placed on the market shall be provided.

GMO DIETARY EXPOSURE ASSESSMENT

The applicant shall determine by appropriate methods the concentrations of the newly expressed proteins, other new constituents and endogenous food and feed constituents, of which the levels have been altered as a result of the genetic modification (for example, due to changes in metabolic pathways) in those parts of the genetically modified plant intended for food or feed use. Expected intake of these constituents shall be estimated taking into account the influences of processing, storage and expected treatment of the food and feed in question, for example, potential accumulation or reduction. In cases where the genetic modification has resulted in an altered level of a natural constituent, or if a new constituent occurs naturally in other food and feed products, the anticipated change in total intake of this constituent shall be assessed considering realistic as well as worst case intake scenarios.

DIETARY EXPOSURE AS PART OF THE RA OF GM FOOD

SCIENCE



- **Comparative dietary exposure frame**: dietary exposure of similar/identical proteins in different foods & dietary exposure to NEPs.

- **Absolute dietary exposure frame**: dietary exposure to NEPs & health based guidance values (risk characterization).

- Dietary exposure in the risk assessment of GM foods

- **How to estimate dietary exposure**

- Human dietary exposure: current situation

- Future

GMO DIETARY EXPOSURE ASSESSEMENT

Field trials – DOSSIER -

Food Terminology FOODEX

EFSA Comprehensive European food consumption database

Concentration values*

Dietary exposure (EXTERNAL)

Food consumption

Scenario 100% replacement

CONCENTRATION DATA

- Substances are analysed in raw primary commodities and consumption data refers to blended (processed) commodities.

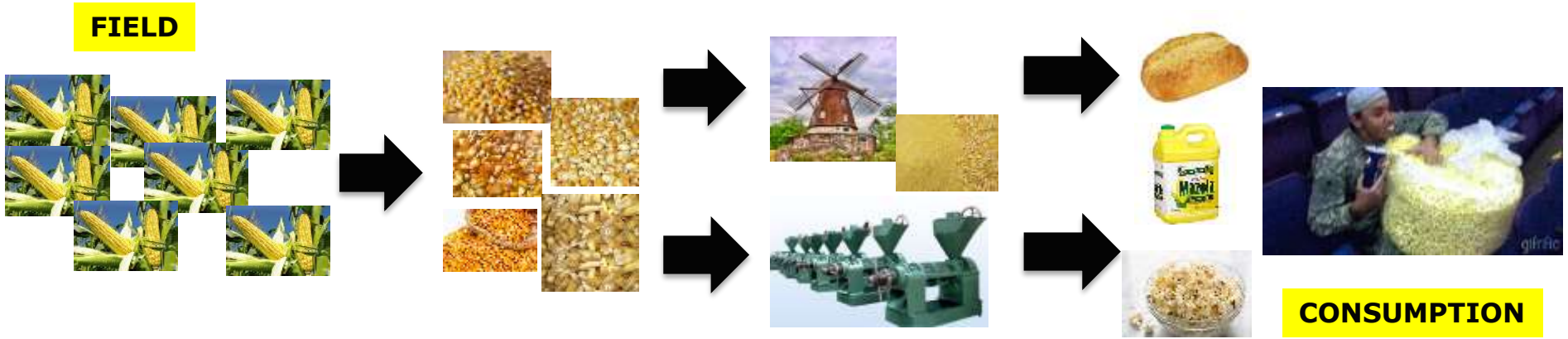
- In most of the cases, very (very) small number of samples available….**representative**??

Which values should be used for dietary exposure estimations ?

Chronic dietary exposure and **acute** dietary exposure

CONCENTRATION DATA

Which values to estimate dietary exposure to GMO components ?



When using **NEP concentrations from RPC** the most realistic scenario is to always* use **MEAN CONCENTRATIONS** for both ACUTE and CHRONIC EXPOSURE

WHICH CONCENTRATION DATA TO BE USED (FROM THE RPC)

- Appropriate material at the representative **growth stage (e.g. maize:** grains **R6/senescence)**

- **Representative of cultivation conditions** (crops treated with the intended herbicide).

- **Mean values** (fresh weight, acute and chronic dietary exposure). *Possibility of differentiating among areas (sites) if significant differences reported

- **LOD/LOQ** to be used for left-censored data (undetected/unquantified) when estimating mean values.

FROM RPC DATA TO PROCESSED COMMODITIES

- **Factors and recipes** linked to processed commodities are also considered.
  - **Recipes** = dilution effect
  - **Reverse yield factors**: **conservative approach***
    - No NEP losses (e.g. due to fermentation)
    - No effect of processing (pH, temperature) as related to the potential hazard of the NEP

- **Protein content in RPC & processed commodities**

FROM RPC DATA TO PROCESSED COMMODITIES

FOODEX LEVEL	FOODEX CODE	FOOD	Amount of raw agricultural commodity to produce 100 grams of processed food (grams)		Comments
4	6220	**Corn grain**	100		100% grain
3	8074	**Popcorn**	100	Puffing=1,08	92,9% grain
4	6268	**Corn flour**	122	Milling= 1,22	100% Corn flour
4	6383	**Corn flakes**	260		100% Corn flakes
4	6384	**Corn flakes and nuts**	190		73% Corn flakes
4	6385	**Corn flakes with honey and nuts**	156	Flaking = 2,6	60% Corn flakes
4	6386	**Corn flakes with honey and sugar**	190		73% Corn flakes
4	6387	**Corn flakes with sugar**	161		62% Corn flakes
4	6445	**Cornmeal porridge**	18		15% corn grain (cornmeal)
4	6271	**Cornmeal**	122	Milling= 1,22	
4	6269	**Corn semolina**	122	Milling= 1,22	
4	6352	**Corn bread**	91	Milling= 1,22	Corn flour 74,3%
3	6336	**Multigrain bread and rolls**	3	Milling= 1,22	Corn flour 2,4 %
3	6407	**Muesli bars**	26	Flaking = 2,6	10% cornflakes
4	6422	**Maize, popped**	108	Puffing=1,08	100% grain
4	6423	**Maize, popped, with sugar**	100		67% corn flour + 33% sugar

CONSUMPTION DATA

- Two main sources of consumption data used in the past in GMO applications:
  - **Pesticide Residues Intake MOdel (PriMo) model**
  - **FAO's Food Balance Sheets (FBSs)**

- It allowed a direct link of the levels of particular constituents measured in RPCs with the consumption data of RPCs

CONSUMPTION DATA

- Some drawbacks associated to the use of PriMo model and/or FBS:
  - FBSs are not appropriate for acute exposure
  - Primo model

    - different methodology to disaggregate the consumption data

    - no possibility to exclude particular foods

EFSA statement in 2015 on the use of the EFSA Comprehensive Consumption database in GMO area

EFSA COMPREHENSIVE CONSUMPTION DATABASE

Number of	
Member States	**25**
Dietary surveys	**60**
Population groups	**132**
Subjects	**119,458**
Consumption records	**12,076,637**

Last update in Aprile 2018

CONSUMPTION DATA –SUMMARY-

- Chronic/acute dietary exposure

- Selection of food commodities relevant for exposure
- Average population and high consumers

- Different age classes across Europe
- Special population groups: vegetarians, lactating women and pregnant women.

- Dietary exposure in the risk assessment of GM foods

- How to estimate dietary exposure

- **Human dietary exposure: current situation**

- Future

CURRENT SITUATION

- Dietary exposure to NEP thoroughly addressed and reported in SO from AP-121 onwards.*

- Information reported in the SO: acute and chronic dietary exposure, only high consumers.

- Information provided by the applicants: **very diverse**

  - Inconsistency between scope and assessment; indistinct use of **expression of results** (fresh weight/dry weight); chronic exposure/acute **dietary exposure** not provided; missing information on **high consumers;** inadequate use of **summary statistics on consumption…**

- Preparation of a document providing guidance to harmonise data needed on exposure estimations.

FOOD CONSUMPTION

EFSA Comprehensive European food consumption database

SUMMARY STATISTICS

Summary food consumption statistics (chronic and acute) available per

- country,
- survey,
- age group (from infants to elderly)
- codified in FoodEx
- in g/day and g/kg bw per day.

USE OF SUMMARY STATISTICS

EFSA Comprehensive European food consumption database

Chronic dietary exposure in the average population

Sum the exposure of all relevant foods obtained by multiplying the average consumption of each food by the mean value reported in the GM crop for the compound of interest (e.g. NEP).

Chronic dietary exposure of high consumers

Sum the high percentile (e.g. 95th) of the most consumed food among only consumers and the average consumption of all the other foods in the whole population, using the mean value reported in the GM crop for the compound.

"Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances" (EFSA, 2011)

Food additives intake model (FAIM) Template (2012)

David R. Tennant (2016) Comprehensive European dietary exposure model (CEDEM) for food additives, Food Additives & Contaminants: Part A, 33:5, 772-781, DOI: 10.1080/19440049.2016.1166898

USE OF SUMMARY STATISTICS

EFSA Comprehensive European food consumption database

Acute dietary exposure for the average population and high consumers.

As we talk about acute exposure, the consumption refers to the amount of food commodity consumed in only one day so the summary statistics for acute consumption should be used. For the rest, same approach as for chronic exposure.

CONSUMPTION DATA

- **Use of EFSA Comprehensive Consumption database (summary statistics) allows…**
  - Assessment **chronic** and **acute** dietary exposure (screening tool)
  - **Average** and **high consumers**
  - **Extensive coverage** of European population
  - Selection of **food commodities relevant for exposure**
  - Possibility of looking at **vulnerable** population groups (based on age, consumption habits, life status)

- Dietary exposure in the risk assessment of GM foods
- How to estimate dietary exposure
- Human dietary exposure: current situation
- **Future**

DOCUMENT ON HUMAN DIETARY EXPOSURE

- **AIM**: To provide guidance on how human dietary exposure to GM constituents should be estimated making the best use of the available information. Description of the information needed for the RA (standardisation)

- **Presentation based on the on-going document.**
- **Publication first quarter of 2019.**

DOCUMENT ON HUMAN DIETARY EXPOSURE

- **Concentration data to be used (mean values, fresh weight, use LOD/LOQ, etc.)**
- **How to best use summary statistics on consumption data**
- **Recipes and factors provided in EFSA website**
- **Information to be provided as part of the submission dossier**

RAW PRIMARY COMMODITY MODEL (RPC MODEL)

Raw Primary Commodities (RPCs)

Reverse yield factors

Individual ingredients

Food as consumed

List of ingredients and proportions

GMO DIETARY EXPOSURE ASSESSEMENT

Field trials – DOSSIER -

Food Terminology FOODEX

EFSA Comprehensive European food database

Raw Primary Commodities (RPC MODEL)

Concentration values*

Dietary exposure (EXTERNAL)

Food consumption

Scenario 100% replacement

TAKE HOME MESSAGES ON DIETARY EXPOSURE

- Dietary exposure to be used on concluding on the safety of the endogenous/new constituents = **representative and accurate levels needed** !!

- Dietary exposure estimations today could be different tomorrow: need of **monitoring** → **different outcome of the RA** → **still SAFE** ?

TAKE HOME MESSAGES ON DIETARY EXPOSURE

- There are **uncertainties** surrounding dietary exposure estimations

Assumptions introduce uncertainty → Overall: **conservative estimations** → **Realistic and representative**

Where can the uncertainty be reduced?

- **Consumption: 100% replacement**
- **Improve representativity of samples ??**
- **Accuracy of the measurements??**
- **Appropriate use of the available data (mean, fresh weight, etc.)**
- **Concentration data on processed foods ??**
- **Processing studies…at the moment only DILUTION due to recipes…**

THANKS!!

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QUESTIONS ?